

# PREVALENCE AND COMPARISON BETWEEN THE EFFICACY OF DIFFERENT TECHNIQUES FOR DIAGNOSIS OF *TOXOPLASMA GONDII* AMONG WOMEN IN ERBIL PROVINCE-IRAQI KURDISTAN

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## Abstract

**Introduction:** Toxoplasmosis is one of the most common social and gynecological problems in Iraq.

**Materials and Methods:** This study investigated the prevalence of *Toxoplasma gondii* among females attending the gynaecological out patient's clinics in Maternity and Rizgary hospitals, several health centers and some private clinics in Erbil-Iraqi Kurdistan, from 16th July 2007 till 15th July 2008, by using different diagnostic methods.

Serological diagnosis was done using LAT, ELISA-IgM and IgG tests and 2ME method, for investigation of *T. gondii* seroprevalence, in relation with residency, marital status, age, education, occupation, gestation, frequency of abortion and infertility.

**Results:** The rate of *Toxoplasma* seropositivity, was higher using LAT 54.46%, followed by ELISA IgG 37.5%, 2ME 14.29% and ELISA IgM 9.13%, and higher rates were recorded among rural inhabitants in comparison with those living in urban areas. The rate of *Toxoplasma* seropositivity was highest among married women, and the age group 47-57 years revealed highest infection rate. The higher rates were indicated among the housewives than the employers.

The pregnant women have higher seroprevalence rate than non-pregnant patients. Women with more than three abortions showed the higher rate of seropositive antibodies.

**Conclusions:** Significant difference was recorded between the techniques and seropositivity of *T. gondii* antibodies which was higher by LAT than ELISA-IgG, ELISA IgM and 2ME.

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**Keywords:** *Toxoplasma gondii*, Prevalence, Diagnostic methods, Erbil-Iraq

## Introduction

Toxoplasmosis is a major public health problem, with a high socioeconomic impact in terms of human suffering including cost of caring for sick, mentally retarded and blind children (1). The parasite is an extremely successful pathogen, responsible for significant morbidity and mortality, especially in congenitally infected and immuno-compromised individuals (2).

Toxoplasmosis can be diagnosed serologically by several tests that depend on the demonstration of *Toxoplasma* antibodies IgM and IgG in serum (3). These tests include the dye test, immunofluorescent study test, latex agglutination test, the enzyme-linked immunosorbent assay (ELISA) and complement fixation test. In recent years, the PCR (polymerase chain reaction) amplification of parasite ribosomal DNA was developed for the detection of single organism in the tissue samples (4).

The study was planned to determine the prevalence of toxoplasmosis in women in Erbil governorate and its relationship with the number of abortion and infertility and to compare between the efficacy of different direct examination and serological tests for diagnosis of *Toxoplasma* seropositivity.

## Materials And Methods

Time and location

Three hundred and three female patients were enrolled in the study, between 16th July 2007 and 15th July 2008, for detection of *Toxoplasma gondii* infection, they were attended to some private

clinics, gynecological out patient's clinics in Maternity and Rizgary hospitals and several health centers in Erbil Governorate.

#### Collection of specimens

Blood samples were withdrawn, from the patients and controls, centrifuged and sera were collected kept at 4-8°C for about 24-48 hours, if longer period was needed, then they were stored in a deep freezing at -20°C for performing serological tests.

#### Serological tests

Four serological tests were used for detection of specific antibody to *Toxoplasma gondii* by the use of Latex Agglutination Test (LAT), IgM and IgG antibodies using ELISA (Enzyme-Linked Immunosorbent Assay) technique and 2 Mercapto Ethanol (2ME).

a. Latex agglutination test (LAT) is performed using toxoplasmosis Latex Test Kits from Plasmatec Laboratory Products Ltd (U.K.).

b. The Toxoplasma IgM ELISA and IgG ELISA were done, using the Genesis Diagnostics (UK) and BioCheck (CA) Toxoplasma IgM kits.

c. 2Mercapto-Ethanol (2ME) done by using the complete kit of 2-Mercapto Ethanol from BioMerieux.

#### Statistical analysis

The statistical analysis was conducted using the software program Statistical Program Social System (SPSS version 13.0). Independent-Samples t-test was used for the biochemical tests for determination of the significant variations. The other remaining data were analyzed using Chi-square test (5).

### Results

Relationship between prevalence of *Toxoplasma gondii*-Ab and residency of the patients using different laboratory methods is shown in Table (1). The rate of *Toxoplasma* seropositivity was higher using LAT 54.46%, followed by ELISA IgG 37.5%, 2ME 14.29% and ELISA IgM 9.13%. Higher rates in the subjects were recorded, in almost all the tests, from rural villages than urban areas, but statistical difference was insignificant.

**Table 1: Relationship between prevalence of *Toxoplasma* Ab and residency of the patients using different laboratory methods.**

Residency	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	×	+	%	×	+	%	×	+	%	×	+	%
Urban	190	103	54.2	137	13	9.49	137	50	36.5	63	7	11.1
Rural	113	62	54.8	71	6	8.45	71	28	39.4	35	7	20.0
Total	303	165	54.4	208	19	9.13	208	78	37.5	98	14	14.2
			6			4			0			9
	$\chi^2=0.012$ df=1 P>0.01			$\chi^2=0.061$ df=1 P>0.01			$\chi^2=0.172$ df=1 P>0.01			$X^2=1.452$ df=1 P>0.0		

The overall marital status-adjusted *T. gondii* seroprevalence, as shown in Table (2), was found among married community higher than single (unmarried) and others (widow, divorced, and separated relationships). Using LAT, the highest seroprevalence rate was among married patients 54.95% followed by single and others (widow, divorced and separated women) 50% and 33.33% respectively, while only married females show seropositivity for toxoplasmosis using ELISA-IgG, 2ME and ELISA-IgM 38.81%, 14.58% and 9.45% respectively.

**Table 2: Seroprevalence of *Toxoplasma gondii* according to the marital status of the patients using different laboratory methods.**

Marital status	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	exam	No. +ve	%	exam	No. +ve	%	exam	No. +ve	%	exam	No. +ve	%
Single	4	2	50.00	2	0	0	2	0	0	1	0	0
Married	293	161	54.95	201	19	9.45	201	78	38.81	96	14	14.5
Others	6	2	33.33	5	0	0	5	0	0	1	0	0
Total	303	165		208	19		208	78		98	14	

Others = Widow, Divorced and Separated Women.

Considering the age group and its relation with the distribution of seropositive *Toxoplasma* antibodies, women of the age group 47-57 years had the highest percentage 66.67% of the positive results of *Toxoplasma* Ab, followed by the age groups 25-35 years 56.93%, 14-24 years 56.48% and 36-46 years 43.64% using LAT. The highest seropositivity rate of *Toxoplasma* antibodies by ELISA-IgM was 13.43% in the age group of 14-24 years, followed by 8.25% and 4.76% in the age groups 25-35 and 36-46 years respectively. While in ELISA-IgG, the age group 47-57 years showed the highest positive rate 50% followed by 14-24 years 46.27%, 25-35 years 34.02% and 36-46 years 30.95%. Using 2ME method, only the age groups 14-24 and 25-35 years were positive for toxoplasmosis 19.15% and 11.9% respectively (table 3).

**Table 3: Seroprevalence of *Toxoplasma gondii* according to the age groups using different laboratory methods.**

Age (year)	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	exam	No. +ve	%	exam	No. +ve	%	exam	No. +ve	%	exam	No. +ve	%
14-24	108	61	56.48	67	9	13.43	67	31	46.27	47	9	19.15
25-35	137	78	56.93	97	8	8.25	97	33	34.02	42	5	11.90
36-46	55	24	43.64	42	2	4.76	42	13	30.95	9	0	0
47-57	3	2	66.67	2	0	0	2	1	50.00	-	-	-
Total	303	165		208	19		208	78		98	14	

Table (4) reveals seroprevalence of toxoplasmosis according to the educational status using various laboratory methods and a statistical association was not found between *T. gondii* seroprevalence and the education level of the women but the disease is more prevalent among women with school education and illiterate patients almost in all the tests and lower seroprevalence was present between women had college education.

**Table 4: Seroprevalence of *Toxoplasma gondii* according to the educational status using different laboratory methods.**

Educational status	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	exam	No. +ve	%	exam	No. +ve	%	exam	No. +ve	%	exam	No. +ve	%
Illiterate	60	33	55.00	41	4	9.76	41	15	36.59	13	3	23.08
School	194	110	56.70	128	13	10.16	128	54	42.19	72	11	15.28
University	49	22	44.90	39	2	5.13	39	9	23.08	13	0	0
Total	303	165		208	19		208	78		98	14	
	$\chi^2=2.206$ df=2 P>0.01			$\chi^2=0.934$ df=2 P>0.01			$\chi^2=4.464$ df=2 P>0.01			$\chi^2=3.045$ df=2 P>0.01		

Table (5) illustrates the seroprevalence of toxoplasmosis according to the occupation using four laboratory techniques and the study revealed that the prevalence of toxoplasmosis increases among the housewives than the employers, but was significantly different by LAT. The higher positive infection rates were investigated among the housewives 57.79% by using LAT, 39.13% ELISA IgG, 16.05% 2ME and 10.56% ELISA IgM, than the employers 40.68%, 31.91%, 5.882% and 4.26% respectively.

**Table 5: Seroprevalence of *Toxoplasma gondii* according to the occupation using different laboratory methods.**

Occupation	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	x	a	m	o	+	%	x	a	m	o	+	%
Housewife	244	141	57.79	161	17	10.56	161	63	39.13	81	13	16.05
Employer	59	24	40.68	47	2	4.26	47	15	31.91	17	1	5.882
Total	303	165	54.46	208	19	9.13	208	78	37.50	98	14	14.29
	$\chi^2=5.607$ *			$\chi^2=1.742$			$\chi^2=0.808$			$\chi^2=1.186$		
	df=1			df=1			df=1			df=1		
	P<0.01			P>0.01			P>0.01			P>0.01		

\* Significant

Table (6) clarifies the significant relationship between seropositive *Toxoplasma* antibodies in regard to gestation using various laboratory methods. Higher seroprevalence of toxoplasmosis, using different serological tests, was found in pregnant women, in whom 66.02% were positive for LAT, 48.39% of the women were IgG-positive, indicating previous maternal infection, 14.29% for 2ME and 11.29% were positive for anti-*Toxoplasma* IgM antibody.

**Table 6: Frequency of *Toxoplasma* seropositive Ab among pregnant and non-pregnant women using different laboratory methods.**

Gestation	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	No. examined	No. +ve	%	No. examined	No. +ve	%	No. examined	No. +ve	%	No. examined	No. +ve	%
Pregnant	103	68	66.02	62	7	11.29	62	30	48.39	42	6	14.29
Non-pregnant	190	93	48.95	139	12	8.63	139	48	34.53	54	8	14.81
	$\chi^2=8.544$ *						df=1			P<0.05		

\* Significant

Regarding the frequency of abortion, the higher rate of seropositive *Toxoplasma* antibodies was found in sera of women with more than 3 abortions by the diagnostic tests (Table 7). The distribution of *Toxoplasma* seropositivity in women with more than 3 abortions was 100% rate of seropositivity using 2ME method, 75% by LAT, 50% for IgG antibodies and 0% for IgM type antibodies specific for *T. gondii*, but statistical analysis appears no significant difference.

**Table 7: Relationship between number of abortion and seropositive *Toxoplasma* Ab using different laboratory methods.**

Number of abortion	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	No. examined	No. +ve	%	No. examined	No. +ve	%	No. examined	No. +ve	%	No. examined	No. +ve	%
Single	63	30	47.62	46	5	10.87	46	17	36.96	18	2	11.11
Double	29	15	51.72	20	1	5.00	20	8	40.00	8	2	25.00
Triple	14	8	57.14	11	1	9.09	11	5	45.45	8	2	25.00
> 3	8	6	75.00	2	0	0	2	1	50.00	1	1	100.0
Total	114	59	51.75	79	7	8.86	79	31	39.24	35	7	20.00
	$\chi^2=2.326$			$\chi^2=0.794$			$\chi^2=0.388$			$\chi^2=5.139$		
	df=3			df=3			df=3			df=3		
	P>0.01			P>0.01			P>0.01			P>0.01		

Table (8) explores the relationship between *Toxoplasma* infection and infertility by determination the level of anti-*Toxoplasma gondii* antibodies in sera of women from the studied group

and the results revealed that the positive result of *Toxoplasma* infection was higher 52.33% using LAT, followed by ELISA-IgG 39.62%, 2ME 15% and ELISA-IgM 9.433% respectively.

In LAT and ELISA tests (IgG and IgM type antibodies), the positive serum samples for *Toxoplasma* infections were more prevalent in women suffering from primary infertility 53.19%, 46.43% and 14.29% than secondary infertility 51.28%, 32% and 4% respectively, while in 2ME, the seropositivity rate in women with secondary infertility was slightly higher than those with primary type of infertility 15.79% and 14.29% respectively. Statistical difference was insignificant.

**Table 8: Seroprevalence of *Toxoplasma gondii* according to the infertility of the patients using different laboratory methods.**

Infertility	Latex agglutination			ELISA-IgM			ELISA-IgG			2ME		
	No. examined	No. +ve	%	No. examined	No. +ve	%	No. examined	No. +ve	%	No. examined	No. +ve	%
Primary	47	25	53.19	28	4	14.29	28	13	46.43	21	3	14.29
Secondary	39	20	51.28	25	1	4.00	25	8	32.00	19	3	15.79
Total	86	45	52.33	53	5	9.433	53	21	39.62	40	6	15
	$\chi^2=0.350$ df=1 P>0.01			$\chi^2=1.635$ df=1 P>0.01			$\chi^2=0.484$ df=1 P>0.01			$\chi^2=0.018$ df=1 P>0.01		

Table (9) shows comparison between efficacies of using four laboratory methods in determination of seropositivity of *Toxoplasma gondii* antibodies in 98 patients. LAT showed the highest rate 48.98% followed by 45.92%, 16.33% and 14.29% with ELISA-IgG, ELISA-IgM and 2ME respectively.

**Table 9: Comparison between efficacies of using four laboratory methods in determination of seropositivity of *T. gondii* Ab in 98 patients.**

Test	No. +ve	%
Latex agglutination	48	48.98
ELISA-IgM	16	16.33
ELISA-IgG	45	45.92
2ME	14	14.29
$\chi^2=47.331^*$ df=3 P<0.01		

\* Significant

The sera of 303 women were examined by Latex agglutination test, 165 were seropositive, and the rate of infection with toxoplasmosis was 54.46%. Out of 208 serum sample tested by ELISA-IgM and IgG, 19 and 78 were seropositive respectively with the rate of seropositivity 9.134% and 37.5% respectively. While among 98 cases tested by 2ME method, only 14 were positive and the rate was 14.29%. The Latex agglutination method revealed the high rate of infection followed by ELISA-IgG, 2ME and ELISA-IgM (Table 10).

**Table 10: Comparison between efficacies of using four laboratory methods in determination of seropositivity of *T. gondii* Ab in 303 patients.**

Test	No. examined	No. +ve	%
Latex agglutination	303	165	54.46
ELISA-IgM	208	19	9.134
ELISA-IgG	208	78	37.50
2ME	98	14	14.29
$\chi^2=132.7^*$ df=3 P<0.01			

\* Significant

## Discussion

The rate of *Toxoplasma* seropositivity was higher in subjects from rural villages than urban areas, with insignificant statistical difference.

Several studies determined a higher prevalence in rural regions in comparison with urban areas like: Al-Griari (6) in Diyala, Kadir and Khana (7) in Sulaimani and Ali (8) in Kalar. While

others did not observe any difference between rural and urban inhabitants like: Al-Kaysi (9) in Baghdad; Al-Jubori (10) in Kirkuk.

The overall marital status-adjusted *T. gondii* seroprevalence, was found among married community higher than single (unmarried) and others (widow, divorced, and separated relationships). Using LAT, the highest seroprevalence rate was among married patients 54.95% followed by single and others (widow, divorced and separated women) 50% and 33.33% respectively, while only married females show seropositivity for toxoplasmosis using ELISA-IgG, 2ME and ELISA-IgM 38.81%, 14.58% and 9.45% respectively. The reasons for higher incidence in married females may be related to soil exposure since they are more involved with child rearing and women with three children were found to have significantly higher seroprevalence of *T. gondii* infection compared with other women (11).

Considering the age group and its relation with the distribution of seropositive *Toxoplasma* antibodies, women of the age group 47-57 years had the highest percentage 66.67% of the positive results of *Toxoplasma* Ab, using LAT. The highest seropositivity rate of *Toxoplasma* antibodies by ELISA-IgM was 13.43% in the age group of 14-24 years, While in ELISA-IgG, the age group 47-57 years showed the highest positive rate 50%. Using 2ME method, only the age groups 14-24 and 25-35 years were positive for toxoplasmosis 19.15% and 11.9% respectively. The highest rate of *Toxoplasma* seropositivity among the age groups 47-57 years respectively are in agreement with the study of Al-Jubori (10) in Kirkuk, he recorded rate of seropositivity 80% in the age group 51 and above.

Statistical association was not found between *T. gondii* seroprevalence and the education level of the women but the disease is more prevalent among women with school education and illiterate patients almost in all the tests and lower seroprevalence was present between women had college education because increased knowledge results in awareness, which consequently results in changes in risky behavior and decline in infection rates, such finding was evident from results of several studies indicated no significant differences were observed between educated and uneducated women with toxoplasmosis like: Al-Griari (6) in Diyala, Ertug *et al.* (12) and Sert *et al.* (13) in Turkey, While Mohammed (3) found the occurrence of the disease was higher among uneducated people than educated ones. In United States it was found that the seroprevalence was significantly higher among those with education below college level in United States (14).

The study revealed that the prevalence of toxoplasmosis increases among the housewives than the employers, but was significantly different by LAT. The higher rate of infection among the housewives than employers may be due to direct contact with infection sources through handling contaminated raw meat or vegetables; poor hand and kitchen hygiene habits, nutrition habits; drinking raw milk or unfiltered water which was found to increase risk of *Toxoplasma gondii* seropositivity for lower and middle socioeconomic populations (15).

Significantly, higher seroprevalence of toxoplasmosis, using different serological tests, was found in pregnant women, in whom 66.02% were positive for LAT, 48.39% of the women were IgG-positive, indicating previous maternal infection, 14.29% for 2ME and 11.29% were positive for anti-*Toxoplasma* IgM antibody, and the frequency of *T. gondii* IgM immunoglobulin detected in the current study may be not attributed to old infection-a possibility suggested to Gras *et al.* (16) who declared that in up to 27% of pregnant women IgM immunoglobulin levels persist for more than two years, making it difficult to pinpoint the timing of infection.

In comparison of the present findings with results of other studies performed in Iraq and other countries, the prevalence of anti-*Toxoplasma* antibodies was found to be much lower than that recorded by Al-Jubori (10) in Kirkuk 93.22% and 61.02% for pregnant and non-pregnant women respectively using the same techniques, and Al-Griari (6) in Diyala, she found the rate of seropositivity among pregnant women 70.4%; 63% in Mosul (17); but the present rate was higher than that recorded by Kadir *et al.* (18) 36.67% in Kirkuk

The higher rate of seropositive *Toxoplasma* antibodies was found in sera of women with more than 3 abortions by the diagnostic tests. The distribution of *Toxoplasma* seropositivity in women with more than 3 abortions was 100% rate of seropositivity using 2ME method, 75% by LAT, 50% for IgG antibodies and 0% for IgM type antibodies specific for *T. gondii*, but statistical analysis appears no significant difference. These results are in consistence with the findings of other investigators like: Abdel Hafez *et al.*, (19) in Jordan, who found that the prevalence of IgG seropositivity in women with

habitual abortions is two times higher than that in women with normal pregnancies 58.2% vs 26.1% which means that the infection may have long lasting effects which produce multiple abortions. Sahwi *et al.* (20) in Egypt estimated that chronic and not acute toxoplasmosis, most probably is a significant cause of repeated abortion and it may be due to the reactivation of chronic infection of the uterus, when no parasitic medication is given after the first abortion, causing rupture of the tissue cyst as a result of the enlargement in the size of the uterus during pregnancy causing liberation of the organisms and fetal infection and damage leading to pregnancy wastage. The present findings also strongly support the conclusion of several researchers in Iraq and other countries in their investigations that there is no relationship between *Toxoplasma* seropositivity and number of abortion (7, 8, 12 and 21).

In LAT and ELISA tests (IgG and IgM type antibodies), the positive serum samples for *Toxoplasma* infections were more prevalent in women suffering from primary infertility 53.19%, 46.43% and 14.29% than secondary infertility 51.28%, 32% and 4% respectively, while in 2ME, the seropositivity rate in women with secondary infertility was slightly higher than those with primary type of infertility 15.79% and 14.29% respectively. Statistical difference was insignificant, which may be related to sample size or strain of the parasite, but are in agreement with the investigations of several studies like: Janitschke *et al.* (22) in Germany, recorded 49% rate of seropositivity for *Toxoplasma*-IgG antibodies and 6.78% IgM antibodies.

The sera of 303 women were examined by Latex agglutination test, 165 were seropositive, and the rate of infection with toxoplasmosis was 54.46%. Out of 208 serum sample tested by ELISA-IgM and IgG, 19 and 78 were seropositive respectively with the rate of seropositivity 9.134% and 37.5% respectively. While among 98 cases tested by 2ME method, only 14 were positive and the rate was 14.29%. The Latex agglutination method revealed the high rate of infection followed by ELISA-IgG, 2ME and ELISA-IgM.

The compound 2 mercapto-ethanol (2ME) is used as reductant of sulphur dioxide which binds the five units of IgM antibody and thus leads to its destruction and lysis. The addition of this compound to serum, the IgM will lose its ability to agglutinate antigen while IgG antibodies will remain unaffected, therefore it is possible to diagnose the chronic form of infection (23).

The prevalence of positive 2ME in this study 14.29% was higher than that recorded in Kirkuk 7.83% (10) and lower than that reported in Erbil city 54.45% (24). These findings could be explained by the fact that the group examined consisted of healthy persons, and IgG positive persons were infected with latent toxoplasmosis without a persistence of IgM antibodies after acute infection in the past.

It is recommended to screen young females of reproductive age before marriage for *Toxoplasma* antibodies using ELISA (IgM and IgG) and treat them.

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